**Supplementary material and methods**

**Plant diversity effects on herbivory are mediated by soil biodiversity and plant chemistry**

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*Secondary metabolome sampling and sample processing*

All samples were processed, extracted, and analysed according to Ristok *et al.* (2019)1 with slight changes. We extracted 20 mg dried ground plant tissue of each sample in 1 mL of extraction buffer (methanol / 50 mM acetate buffer, pH 4.8; 50 / 50 [v/v]). The samples were homogenized for 5 min at 30 Hz using a ball mill (Retsch mixer mill MM 400), and subsequently centrifuged (20000 g, 10 min, 4°C). The supernatant was collected in a 2 mL Eppendorf tube. We repeated the extraction procedure with the remaining pellet and combined the supernatant with the first one. We centrifuged (20000 g, 5 min, 4°C) all extracts, transferred 200 µL to an HPLC vial and added 800 µL extraction buffer, resulting in a 1:5 dilution.

We performed chromatographic separation of all diluted extracts by injecting 2 µL on a Thermo Scientific Dionex UltiMate 3000 (Thermo Scientific Dionex, Sunnyvale, USA) UPLC unit, equipped with a C18 column (Acclaim RSLC 120 C18, 2.2 µm, 120 Å, 2.1 x 150 mm, Thermo Fisher Scientific). We applied the following binary elution gradient at a flow rate of 0.4 mL min-1 and a column temperature of 40°C: 0 – 2 min, 95% A (water and 0.05% formic acid), 5% B (acetonitrile and 0.05% formic acid); 2 – 12 min, 5 to 50% B; 12 – 13 min, 50 to 95% B; 13 – 15 min, 95% B; 15 – 16 min, 95 to 5% B; 16 – 20 min, 5% B.

Metabolites were analysed on a liquid chromatography quadrupole time-of-flight mass spectrometer (LC-qToF-MS; Bruker maXis impact HD; Bruker Daltonik, Bremen, Germany) with an electrospray ionization source operated in negative mode. Instrument settings were as follows: capillary voltage, 2500 V; nebulizer, 2.5 bar; dry gas temperature, 220°C; dry gas flow, 11 L min-1; scan range, 50 – 1500 m/z; acquisition rate, 3 Hz. We used sodium formate clusters (10 mM solution of NaOH in 50 / 50% [v/v] isopropanol / water containing 0.2% formic acid) to perform mass calibration.

*LC-MS data processing and metabolite prediction*

We followed the LC-MS data processing protocol described in Ristok *et al.* (2019)1 with minor changes. We converted the LC-qToF-MS raw data to the mzXML format by using the CompassXport utility of the DataAnalysis vendor software. Subsequently, we trimmed each data file by excluding the same non-informative regions at the beginning and end of each run using the msconvert function of ProteoWizard v3.0.10095 2. We performed peak picking, feature alignment, and feature group collapse in R v3.3.3 3 using the Bioconductor4 packages ‘xcms’ 5–7 and ‘CAMERA’ 8. We used the following ‘xcms’ parameters: peak picking method “centWave” (snthr = 10; ppm = 5; peakwidth = 4, 10); peak grouping method “density” (minfrac = 0.5; bw = 6, 3; mzwid = 0.01); retention time correction method “symmetric”. We used ‘CAMERA’ to annotate adducts, fragments, and isotope peaks with the following parameters: extended rule set (https://gitlab.com/R\_packages/chemhelper/-/tree/master/inst/extdata); perfwhm = 0.6; calcIso = TRUE; calcCaS = TRUE, graphMethod = lpc. Lastly, we collapsed each annotated feature group, hereafter referred to as ‘metabolite’ which is described by mass-to-charge ratio (m/z) and retention time (rt), using a maximum heuristic approach. In detail, this means that the intensity values of the feature that most often displayed the highest intensity across all samples represents the feature group. We performed pre-processing with ‘xcms’ and ‘CAMERA’ 8 separately for each species and sampling season. We merged all created feature lists by retention time and mass-to-charge values. For each feature, we allowed for a retention time window of 10 seconds and a mass deviation of 5 ppm.

*Phospholipid fatty acid analysis*

We analysed all samples on a gas chromatograph (GC-FID; Clarus 680, Perkin Elmer) equipped with a SR-2560 column (0.25 mm x 100 m, 0.2µm, Sigma-Aldrich) and helium as carrier gas. We applied the following temperature gradient: 0-5 min, 100°C column temperature; 5-40 min, 100°C to 240°C; 40-50 min, 240°C. The dry gas flow was set to 1.4 ml min-1.

*Statistical analysis*

We analysed our data in the statistical software R v3.5 3 using (<http://www.r-project.org>) the packages, ‘vegan’ 9, ‘lme4’ 10, ‘lmerTest’ 11, ‘plspm’ 12, and ‘effects’ 13.

We calculated multi-response permutation procedures (MRPP) on log + 1-transformed data to test for significant differences in the metabolite composition between the different sown plant species richness levels. We used the Bray-Curtis MRPP dissimilarity matrix. Each analysis was species-specific and sampling season-specific and permuted 10000 times. We were not able to calculate pairwise comparisons of the metabolite composition between plants grown in monoculture and in the highest diversity plot. This is due to the experimental design and its limitations. For each species, only one monoculture plot was present. In addition, there was only one 8-species plot. This meant there were not enough replicates to run MRPP and, as such, the pairwise comparisons between monoculture and the 8-species plot were excluded from the analyses.

We calculated two metrics of metabolite diversity: (a) the richness of secondary metabolites, *i.e.* the number of metabolites within a plant individual; and (b) the Shannon diversity of secondary metabolites, *i.e.* the abundance-weighed diversity of metabolites expressed as the exponential of the Shannon-Weaver index14 based on plant individual-level metabolite intensities.

Moreover, for each trait considered in the design of the Trait-Based-Experiment, we calculated community-weighted mean (CWM) trait values15. Here, we based the calculations on the relative species-specific cover for each plant community.

To test for the effect of sown plant species richness or CWM trait values on the richness or Shannon diversity of secondary metabolites, we calculated linear mixed effects models. We fitted either the richness or the Shannon diversity of secondary metabolites as response variables. As predictor variables, we fitted sampling season (categorical; August 2015 or May 2016), plant functional group identity (categorical; grass or forb), and either sown plant species richness (metric; 1, 2 , 4 or 8) or each of the CWM traits separately (metric, scaled), as well as the three-way interaction. We fitted plot nested in block and species identity as independent random effects. Model simplification was achieved by model comparison using Akaike Information Criterion (AIC). All linear mixed effects models were based on restricted-maximum likelihood estimation and Type I analysis of variance with Satterthwaite approximation for degrees of freedom.

In order to ensure the robustness of our linear mixed effects models, we calculated a second set of models with our predictor variables fitted in the following order: either sown plant species richness or each of the CWM traits separately or either CMS\_PCA1 or CMS\_PCA2, plant functional group identity, and sampling season. Since the model outcomes were similar to our first set of models, we decided to present the result of our first set of models in the manuscript.

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